

LISTING OF CLAIMS

1. *(amended)* A method of determining the copy number (CN) of a first nucleotide sequence I (NucSeqI) in a sample using an amplification technique, said method comprising the steps of:

- (1) adding to the sample nucleotides, primers, polymerase and optionally, any additional ~~further reagents, if any, required for the amplification technique used to the sample,~~
- (2) performing one or more amplification cycles to amplify the ~~nucleotide sequence~~ NucSeqI ~~for which the copy number has to be determined;~~

wherein the sample comprises ~~contains a chromosomal~~ chromosome-derived second nucleotide sequence II (NucSeqII), and the following amplification steps are carried out:

- (a) ~~the first nucleotide sequence~~ NucSeqI is amplified,
- (b) ~~the second nucleotide sequence~~ NucSeqII is amplified,
- (c) a third nucleotide sequence I' (NucSeqI') corresponding to ~~the first nucleotide sequence~~ NucSeqI and present in a control sample is amplified at various multiple dilutions, and
- (d) a fourth nucleotide sequence II' (NucSeqII') corresponding to ~~the second nucleotide sequence~~ NucSeqII and present in a control sample is amplified at multiple various dilutions,

wherein the ratio of the concentration[[s]] of ~~nucleotide sequence~~ NucSeqI' and the concentration of NucSeqII' is known,

wherein ~~the amplification[s] of the third and fourth nucleotide sequences~~ NucSeqI' and NucSeqII' at multiple various dilutions results in the generation of ~~allows~~ standard curves SC_i, SC_I and SC_{II}, respectively ~~with i being I or II to be made,~~ such that the concentrations of NucSeqI and NucSeqII are determined by using the respective standard curves SC_I and SC_{II} ~~SC_i~~, such that ~~the relative concentrations allows the relative copy number CN of sequence~~ NucSeqI relative to (versus nucleotide sequence II) NucSeqII is to be determined using the formula

$$CN = \frac{\text{Conc-I}_{SC_I} \{H\}_{SC_I}}{\text{Conc-II}_{SC_{II}} \{H\}_{SC_{II}}}$$

wherein

- (i) CN is the relative copy number of NucSeqI relative to over NucSeqII) in the sample;
- (ii) Conc-I_{SC_I} is the concentration of NucSeqI determined using standard curve SC_I; and

~~{H_{SCII}}~~ (iii) Conc-II_{SCII} is the concentration of NucSeqII determined using standard curve SC_{II}, and

wherein:

at least one pair of amplification reactions ~~selected from chosen from i)~~ (a) and (b), and ~~[[ii]]~~ (c) and (d) is performed in a single container and monitored spectrophotometrically during amplification, and

~~the third nucleotide sequence NucSeqI'~~ and ~~fourth nucleotide sequence NucSeqII'~~ are localized reside on a single vector.

2. (amended) A method according to claim 1, ~~wherein characterized in that the~~ an absolute copy number is determined by multiplying ~~the copy number~~ CN by the absolute copy number of copies of sequence NucSeqII per cell.

3. (amended) A method according to claim 1 ~~or 2, wherein characterized in that~~ at least two ~~and also more~~ different ~~third nucleotide sequences NucSeqI'~~ sequences used for measuring a corresponding number of different ~~first nucleotide sequences NucSeqI~~ sequences are localized ~~reside on~~ a single vector.

4. (amended) A method according to claim 1 ~~any of the preceding claims, characterized in that wherein~~ the sequences of the ~~first nucleotide sequence NucSeqI~~ is the same as ~~and the third nucleotide sequence NucSeqI'~~ are the same.

5. (amended) A method according to claim 1 ~~any of the preceding claims characterized in that wherein~~ the sequences of the ~~second nucleotide sequence NucSeqII~~ is the same as ~~and the fourth nucleotide sequence NucSeqII'~~ are the same.

6. (new) A method according to claim 2, wherein at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI are localized on a single vector.

7. (new) A method according to claim 2 wherein the sequences of NucSeqI and the NucSeqI' are the same.

8. (new) A method according to claim 3 wherein the sequences of NucSeqI and the NucSeqI' are the same.

9. (new) A method according to claim 6 wherein the sequences of NucSeqI and the NucSeqI' are the same.

10. (new) A method according to claim 2 wherein the sequences of NucSeqII and the NucSeqII' are the same.

11. *(new)* A method according to claim 3 wherein the sequences of NucSeqII and the NucSeqII' are the same.

12. *(new)* A method according to claim 4 wherein the sequences of NucSeqII and the NucSeqII' are the same.

13. *(new)* A method according to claim 6 wherein the sequences of NucSeqII and the NucSeqII' are the same.

14. *(new)* A method according to claim 7 wherein the sequences of NucSeqII and the NucSeqII' are the same.

15. *(new)* A method according to claim 8 wherein the sequences of NucSeqII and the NucSeqII' are the same.

16. *(new)* A method according to claim 9 wherein the sequences of NucSeqII and the NucSeqII' are the same.